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(FILE 'USPAT' ENTERED AT 11:01:25 ON 15 SEP 93)

L1 12459 S NEUR?  
L2 40769 S DIFFERENTIAT?  
L3 1267 S L1 AND L2  
L4 3134 S GROWTH(A)FACTOR#  
L5 234 S L3 AND L4  
L6 8000 S PROLIFERAT?  
L7 250 S L1 AND L6 AND L4  
L8 155 S L5 AND L7  
L9 2302 S CULTUR? AND L1  
L10 220 S L2 AND L9 AND L4  
L11 47 S L2(5A)PRECURSOR  
L12 12 S L1 AND L11  
L13 3 S L4 AND L12  
L14 0 S NEURAL STEM  
L15 2 S NEURONAL STEM  
L16 35713 S 1-2  
L17 59 S L9 AND PROGENITOR#  
L18 32 S L17 AND L2 AND L4

=> t s6/7/18,40,36,34,24,29,19,15,49,57

6/7/18 (Item 18 from file: 5)  
9588826 BIOSIS Number: 94093826

EFFECTS OF ACIDIC AND BASIC FIBROBLAST GROWTH FACTORS AFGF BFGF ON GLIAL  
PRECURSOR CELL PROLIFERATION AGE DEPENDENCY AND BRAIN REGION SPECIFICITY

ENGELE J; BOHN M C

DEP. ANAT. AND CELL BIOL., UNIV. ULM, ALBERT-EINSTEIN-ALLEE 11, POSTFACH  
4066, 7900 ULM, GER.

DEV BIOL 152 (2). 1992. 363-372. CODEN: DEBIA

Full Journal Title: Developmental Biology

Language: ENGLISH

Acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor  
(bFGF) are present in high levels in most areas of the embryonic rodent  
brain. To begin to understand the role of these growth factors in brain

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development, the effects of aFGF and bFGF on dissociated cell cultures prepared from embryonic and neonatal rat brain were studied. Addition of aFGF and heparin or bFGF alone to serum-free cultures of the dissociated Embryonic Day (E) 14.5 mesencephalon stimulates cell proliferation, as judged by [<sup>3</sup>H]thymidine autoradiography, leading to a maximal 75-fold increase in the total number of cells. This effect is dose-dependent with half-maximal increases at concentrations of about 5-6 ng/ml of aFGF or bFGF and is inhibited by the FGF antagonist HBGF-1U. The effect of aFGF on cell proliferation in cultures prepared from E14.5 mesencephalon is similar to that in cultures prepared from E14.5 cortex. However, in cultures prepared from E14.5 rhombencephalon or diencephalon, the proliferative effect of aFGF is much reduced. In all brain areas studied, the proliferative effect of aFGF declines with increasing age. Immunocytochemical analysis of E14.5 mesencephalic cultures demonstrated that the aFGF-induced increases in cell number is due to the proliferation of A2B5-immunoreactive (IR) glial precursor cells, but not of neuronal precursors, fibroblasts, or microglial cells. Moreover, differentiated glial fibrillary acidic protein-IR astrocytes and 2',3'-cyclic nucleotide 3'-phosphohydrolase-IR oligodendrocytes were not observed in culture continuously treated with aFGF or bFGF, but were observed in high numbers after removal of the growth factors. These results suggest that aFGF and bFGF are potent mitogens for glial precursor cells in all embryonic brain regions, that the magnitude of the effects of aFGF depends on embryonic age and brain region, and that both factors inhibit the differentiation of astrocyte or oligodendrocyte precursors. These observations made in vitro strongly support the hypothesis that FGF play a critical role in gliogenesis and the timing of glial differentiation in the brain.

6/7/40 (Item 40 from file: 5)

6439594 BIOSIS Number: 85040115

ACIDIC AND BASIC FIBROBLAST GROWTH FACTORS PROMOTE STABLE NEURITE  
OUTGROWTH AND NEURONAL DIFFERENTIATION IN CULTURES OF PC12 CELLS

RYDEL R E; GREENE L A

DEP. PHARMACOL., N.Y. UNIV. MED. CENT., 550 FIRST AVE., NEW YORK, N.Y.  
10016.

J NEUROSCI 7 (11). 1987. 3639-3653. CODEN: JNRSD

Full Journal Title: Journal of Neuroscience

Language: ENGLISH

Acidic (aFGF) and basic (bFGF) fibroblast growth factors are well-characterized peptide hormones that have potent angiogenic activity and that are mitogenic for a variety of cell types. The present findings demonstrate that FGFs can reproduce the entire spectrum of rat pheochromocytoma PC12 cell responses previously shown to be elicited by NGF. These include responses that are rapid (cell flattening, enhanced phosphorylation of tyrosine hydroxylase) or delayed (neurite outgrowth, induction of phosphorylated MAP 1.2, regulation of NILE and Thy-1 glycoproteins, cessation of mitosis, elevation of AChE activity), as well as responses that have been shown to be either transcription-independent (neurite regeneration, promotion of survival) or transcription-dependent (priming, regulation of NILE and Thy-1 glycoproteins, elevation of AChE activity). The only responses for which the FGFs and NGF consistently showed quantitative differences were in the rates for neurite initiation and elongation in serum-containing medium. Thus, while all 3 factors promoted the formation of stable neurites, the network of outgrowth elicited by NGF at any given time of treatment was always of greater density. Togari et al. (1985) have previously reported that bFGF can initiate transient neurite formation in PC12 cell cultures. The present observations describe a variety of additional actions of bFGF on a neuronal cell line, and demonstrate that aFGF is capable of mimicking many, if not all, of these actions. These observations thus extend the range of actions that aFGF and bFGF may potentially exert on nerve cells, either during their development, repair, or maintenance. In addition, this work suggests that the PC12 cell line may serve as a useful model system with which to study the mechanism of action of FGFs on neurons. Since all 3 factors appear capable of eliciting the same wide spectrum of responses, molecular events specifically associated with FGFs and NGF in PC12 cells

may prove illuminating of the causal steps involved in neuronal differentiation.

6/7/36 (Item 36 from file: 5)  
6611676 BIOSIS Number: 86078227

MORPHOLOGICAL DIFFERENTIATION OF EMBRYONIC RAT SYMPATHETIC NEURONS IN  
TISSUE CULTURE II. SERUM PROMOTES DENDRITIC GROWTH

BRUCKENSTEIN D A; HIGGINS D

DEP. PHARMACOL. THER., FARBER 127, SCH. MED., STATE UNIV. NEW YORK,  
BUFFALO, N.Y. 14214.

DEV BIOL 128 (2). 1988. 337-348. CODEN: DEBIA

Full Journal Title: Developmental Biology

Language: ENGLISH

In the preceding paper, we reported that embryonic rat sympathetic neurons formed axons, but not dendrites, when they were maintained in the absence of serum and nonneuronal cells. To assess the effects of serum-derived factors on cellular morphology, cultures were initially maintained in serum-free medium while nonneuronal cells were eliminated. Subsequently some cultures were chronically exposed either to fetal calf serum (10%) or to a high-molecular-weight ammonium sulfate fraction of serum (P40 material, 500 .mu.g/ml). Phase-control microscopy revealed that serum and P40 material did not alter neuronal survival, but did cause flattening of the somata and fasciculation of processes. When neurons exposed to serum or P40 material were injected with Lucifer Yellow, it was found that the majority (.gtoreq. 90%) had local, tapered processes that could be identified as dendrites by light microscopic criteria. These local processes also exhibited other dendritic characteristics in that (1) they reacted with monoclonal antibodies to nonphosphorylated forms of the M and H neurofilament subunits and to microtubule-associated protein 2; and (2) they had substantial amounts of RNA as determined by [3H]uridine autoradiography. Quantitative measurements of the effects of serum and P40 material on dendritic morphology revealed that (1) an 8-day exposure caused most neurons (> 80%) to form dendrites; (2) neurons typically had more than one dendrite (mean of 4.1 +/- 0.2 dendrites/cell after a 28-day exposure); and (3) the dendrites were relatively short with the maximum extent of the dendritic arbor being 110 +/- 13 .mu.m after 4 weeks. Serum and P40 material did not routinely cause the formation of supernumerary axons, did not alter radial axonal outgrowth from ganglion explants, and did not significantly increase [3H]leucine incorporation. Thus, serum contains a factor (or factors) which selectively stimulates the extension of dendrites, but no axons. If such a factor were operative in situ, it could play an important role in determining the morphology of sympathetic neurons. In examining the mechanism of serum-induced dendritic growth, we found that even high concentrations (5 .mu.g/ml) of nerve growth factor failed to promote dendritic growth in the absence of serum; thus, nerve growth factor by itself is not a sufficient condition for the extension of dendrites. It was also observed that the percentage of cells forming dendrites in either serum-free or serum-containing media was unaffected by (1) agonists and antagonists known to bind to various cholinergic and adrenergic receptors; or (2) agents (25 mM K+, tetrodotoxin) known to affect electrical activity in vitro. Thus, serum-induced dendritic growth does not require normal electrical activity or afferent input.

6/7/34 (Item 34 from file: 5)  
7057305 BIOSIS Number: 87117826

INFLUENCE OF EPIDERMAL GROWTH FACTOR ON THE MATURATION OF FETAL RAT BRAIN  
CELLS IN AGGREGATE CULTURE AN IMMUNOCYTOCHEMICAL STUDY

MONNET-TSCHUDI F; HONEGGER P

INSTITUT DE PHYSIOLOGIE, UNIVERSITE DE LAUSANNE, 7 RUE DU BUGNON, CH-1005  
LAUSANNE, SWITZERLAND.

DEV NEUROSCI 11 (1). 1989. 30-40. CODEN: DENED

Full Journal Title: Developmental Neuroscience

Language: ENGLISH

Maturation of astrocytes, neurons, and oligodendrocytes was studied in serum-free aggregating cell cultures of fetal rat telencephalon by an immunocytochemical approach. Cell type-specific immunofluorescence staining

was examined by using antibodies directed against glial fibrillary acidic protein (GFAP) and vimentin, two astroglial markers; neuron-specific enolase (NSE) and neurofilament (NF), two neuronal markers, and galactocerebroside (GC), an oligodendroglial marker. It was found that the cellular maturation in aggregates is characterized by distinct developmental increases in immunoreactivity for GFAP, vimentin, NSE, NF, and GC, and by a subsequent decrease of vimentin-positive structures in more differentiated cultures. These findings are in agreement with observations in vivo, and they corroborate previous biochemical studies of this histotypic culture system. Treatment of very immature cultures with a low dose of epidermal growth factor (EGF, 5 ng/ml) enhanced the developmental increase in GFAP, NSE, NF and GC immunoreactivity, suggesting an acceleration of neuronal and glial maturation. In addition, EGF was found to alter the cellular organization within the aggregates, presumably by influencing cell migration.

6/7/24 (Item 24 from file: 5)  
8650090 BIOSIS Number: 92115090

BASIC FIBROBLAST GROWTH FACTOR REGULATES THE ABILITY OF ASTROCYTES TO SUPPORT HYPOTHALAMIC NEURONAL SURVIVAL IN-VITRO  
PETROSKI R E; GRIERSON J P; CHOI-KWON S; GELLER H M  
GRADUATE SCH., RUTGERS UNIV., PISCATAWAY, N.J. 08854.  
DEV BIOL 147 (1). 1991. 1-13. CODEN: DEBIA  
Full Journal Title: Developmental Biology  
Language: ENGLISH

The putative neurotrophic effects of basic fibroblast growth factor (bFGF) were tested on embryonic hypothalamic neurons in dissociated cell culture. Basic FGF dramatically increased the survival of embryonic hypothalamic astrocytes plated on a poly-L-lysine (PLL) substrate.. Basic FGF treatment also increased the number of hypothalamic neurons surviving in vitro; however, no neurotrophic effects were observed when astrocyte proliferation was prevented by using serum-free N2 medium or by using the mitotic inhibitor cytosine arabinoside. In contrast to effects when PLL was used as a substrate, bFGF reduced the survival of hypothalamic neurons plated on a confluent, contact-inhibited monolayer of astrocytes. This effect appears to be due to the direct actions of bFGF on astrocytes: treatment of confluent astrocytes with 5 ng/ml bFGF caused the protoplasmic astrocytes to develop a fibrillar morphology and reduced the ability of the astrocyte monolayer to promote neuronal survival after a further 24 hr in bFGF-free medium. It is concluded that in addition to its mitogenic effects, bFGF acts as a differentiation factor for protoplasmic astrocytes in vitro, and these morphological and functional changes may reflect the process of normal astrocytic development and response to brain injury in vivo.

6/7/29 (Item 29 from file: 5)  
8132147 BIOSIS Number: 91053147

NERVE GROWTH FACTOR ENHANCES NEURITE ARBORIZATION OF ADULT SENSORY NEURONS A STUDY IN SINGLE-CELL CULTURE  
YASUDA T; SOBUE G; ITO T; MITSUMA T; TAKAHASHI A  
DIV. NEUROL., FOURTH DEP. INTERNAL MEDICINE, AICHI MEDICAL UNIV., NAGAKUTE, AICHI 480-11, JPN.  
BRAIN RES 524 (1). 1990. 54-63. CODEN: BRREA  
Full Journal Title: Brain Research  
Language: ENGLISH

Nerve growth factor (NGF) is a well-established trophic factor of sympathetic and sensory neurons during development. NGF is, however, little known to be required for the maintenance or regulation of differentiated phenotypes of matured peripheral neurons. Since trophic factors, including NGF, are currently known to be secreted by non-neuronal cells, like Schwann cells and fibroblasts, a highly pure-neuron culture is required to assess the direct action of trophic factors on neurons. We have developed a single-neuron culture from neonatal and adult rat dorsal root ganglia in serum-free conditions, and estimated the primary effect of NGF on the morphological geometry of sensory neurons. We found that NGF promoted the neurite length of neonatal sensory neurons rather than promoting

arborization (branching of neurites), while in adult matured neurons NGF significantly enhanced neurite arborizations, rather than the maximal neurite extension, distance from the cell soma to the maximum margin of the territory of neurite extension. Total neurite length, the summed length of all neurites per neuron was significantly increased by NGF in both neonatal and adult neurons. NGF also increased the size of neuronal soma independent of neuronal maturation. Neonatal sensory neurons tended to die in 1 week despite the presence of NGF. In contrast, some adult sensory neurons were alive for more than 2 weeks in the absence of NGF. These results indicate that NGF more than simply accelerates a pre-existing developmental program in the matured stage, and that the promotion of neurite arborization by NGF in adult sensory neurons suggests that NGF may have some role in peripheral nerve regeneration via promotion of axonal sprouting.

6/7/19 (Item 19 from file: 5)  
9577201 BIOSIS Number: 94082201

ACIDIC AND BASIC FIBROBLAST GROWTH FACTORS IN ADULT RAT HEART MYOCYTES  
LOCALIZATION REGULATION IN CULTURE AND EFFECTS ON DNA SYNTHESIS

SPEIR E; TANNER V; GONZALEZ A M; FARRIS J; BAIRD A; CASSCELLS W  
NATL. INST. HEALTH, BUILD. 10, ROOM 7B-15, BETHESDA, MD. 20892, USA.  
CIRC RES 71 (2). 1992. 251-259. CODEN: CIRUA  
Full Journal Title: Circulation Research  
Language: ENGLISH

Basic fibroblast growth factor (bFGF) and acidic fibroblast growth factor (aFGF) are involved in the induction of embryonic mesoderm, angiogenesis, neuronal differentiation, and proliferation and survival of many cell types. In cardiac myocytes their roles are not well understood. Effects of fibroblast growth factors on reexpression of fetal actin genes have been reported. In freshly isolated adult rat cardiac myocytes, bFGF mRNA was not detectable by in situ hybridization, although the cells contained significant amounts of bFGF and aFGF as quantified by radioimmunoassays, mitogen assays with immunoneutralization, and Western blotting. After culturing, bFGF mRNA was detected (aFGF mRNA was not studied), and the cells contained 2.5-fold more bFGF and 60% more aFGF than freshly isolated cells. The FGFs were not found in conditioned medium. They were localized, especially in cultured cells, to the nucleus. Cultured myocytes bound fourfold more <sup>125</sup>I-FGF than freshly isolated cells and expressed the fibroblast growth factor R-1 (flg) gene. The addition of bFGF or aFGF in serum-free medium to pure populations of myocytes (after 10 days in culture, at which time they are spread, beating, and multinucleated) led to increased thymidine incorporation. Expression of fibroblast growth factors and fibroblast growth factor receptors by adult myocytes that survive the shock and "dedifferentiation" of culturing may contribute to DNA synthesis and, by analogy, to other cell types, the regulation of ribosomal and actin genes, and to cell survival. These possibilities and their in vivo relevance will require further study.

6/7/15 (Item 15 from file: 5)  
9618634 BIOSIS Number: 94123634

DIFFERENTIATION FACTORS INCLUDING NERVE GROWTH FACTOR FIBROBLAST GROWTH  
FACTOR AND INTERLEUKIN-6 INDUCE AN ACCUMULATION OF AN ACTIVE RAS GTP  
COMPLEX IN RAT PHEOCHROMOCYTOMA PC12 CELLS

NAKAFUKU M; SATOH T; KAZIRO Y  
DNAX RES. INST. MOLECULAR CELLULAR BIOL., 901 CALIFORNIA AVE., PALO ALTO,  
CALIF. 94304.

J BIOL CHEM 267 (27). 1992. 19448-19454. CODEN: JBCHA  
Full Journal Title: Journal of Biological Chemistry  
Language: ENGLISH

Ras has been thought to be involved in neuronal differentiation of rat pheochromocytoma PC12 cells. PC12 cells are immature adrenal chromaffin-like cells which undergo differentiation to sympathetic neuron-like cells in response to nerve growth factor (NGF). Fibroblast growth factor (FGF) and interleukin (IL)-6 can also induce differentiation of PC12 cells. In this paper, we report that NGF, FGF, and IL-6 induce an accumulation of an active Ras .cntdot. GTP complex. In the serum-starved culture of PC12 cells, 6% of the Ras protein was complexed with GTP. Upon

stimulation with NGF, the percentage of Ras .cntdot. GTP increased to 24% after 2 min, and the high level of Ras .cntdot. GTP was maintained for at least 16 h. On the other hand, the activation of Ras by FGF and IL-6 showed distinct kinetics; about 3-fold increase of Ras .cntdot. GTP was detected at 10 min, and afterward, the level returned to the basal level within 60 min. These observations provide direct evidence that activation of Ras is involved in signal transduction from these differentiation factors. In addition, it was found that growth factors, including epidermal growth factor, insulin, and insulin-like growth factor-I, and a tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), can also activate Ras under the same conditions. A tyrosine kinase-specific inhibitor, genistein, inhibited the increase of Ras .cntdot. GTP induced by NGF and other factors. On the other hand, down-regulation of protein kinase C (PKC) by prolonged treatment with TPA, which sufficiently blocked TPA-induced Ras activation, did not abolish the formation of Ras .cntdot. GTP by NGF. These results suggest that tyrosine kinases rather than PKC play a major role in the NGF-induced activation of Ras in PC12 cells.

6/7/49 (Item 49 from file: 5)  
4093352 BIOSIS Number: 76043203

NERVE GROWTH FACTOR MEDIATED DIFFERENTIATION OF A NERVE CELL LINE  
CULTURED IN A HORMONE SUPPLEMENTED SERUM-FREE MEDIUM

HATANAKA H  
DEP. NEUROCHEM., MITSUBISHI-KASEI INST. LIFE SCI., MACHIDA-SHI, TOKYO  
194.

DEV BRAIN RES 6 (3). 1983. 243-250. CODEN: DBRRD  
Full Journal Title: Developmental Brain Research  
Language: ENGLISH

In newly-isolated subclone of [rat pheochromocytoma cell line] PC12, designated h, nerve growth factor (NGF) is known to cause an increase of tyrosine hydroxylase (TH) activity concomitantly with that of choline acetyltransferase (CAT) activity. When the PC12h cells were cultured and maintained for several generations in a hormone-supplemented serum-free medium, only TH activity was selectively enhanced by NGF, while CAT and glutamic acid decarboxylase activities remained unaffected. PC12h cells cultured in serum-free medium could extend long neurites in response to NGF. The loss of the NGF-mediated increase of CAT activity in PC12h cells cultured in serum-free medium was fully restored upon re-exposure to serum. The NGF-mediated increase of TH activity in PC12h cells cultured in serum-free medium was additive to that increased by an application of 10<sup>-5</sup>-10<sup>-10</sup> M dexamethasone. PC12h cells cultured in chemically-defined serum-free medium, having responses to NGF identical to sympathetic neurons, will be a useful model for elucidating the molecular mechanism(s) of NGF-mediated neuronal differentiation.

6/7/57 (Item 4 from file: 155)  
08268895 92406895

Differentiation factors, including nerve growth factor, fibroblast growth factor, and interleukin-6, induce an accumulation of an active Ras.GTP complex in rat pheochromocytoma PC12 cells.

Nakafuku M; Satoh T; Kaziro Y  
DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California 94304-1104.

J Biol Chem Sep 25 1992, 267 (27) p19448-54, ISSN 0021-9258  
Journal Code: HIV

Languages: ENGLISH  
Document type: JOURNAL ARTICLE

Ras has been thought to be involved in neuronal differentiation of rat pheochromocytoma PC12 cells. PC12 cells are immature adrenal chromaffin-like cells which undergo differentiation to sympathetic neuron-like cells in response to nerve growth factor (NGF). Fibroblast growth factor (FGF) and interleukin (IL)-6 can also induce differentiation of PC12 cells. In this paper, we report that NGF, FGF, and IL-6 induce an accumulation of an active Ras.GTP complex. In the serum-starved culture of PC12 cells, 6% of the Ras protein was complexed with GTP. Upon stimulation with NGF, the percentage of Ras.GTP increased to 24% after 2 min, and the

high level of Ras.GTP was maintained for at least 16 h. On the other hand, the activation of Ras by FGF and IL-6 showed distinct kinetics; about 3-fold increase of Ras.GTP was detected at 10 min, and afterward, the level returned to the basal level within 60 min. These observations provide direct evidence that activation of Ras is involved in signal transduction from these differentiation factors. In addition, it was found that growth factors, including epidermal growth factor, insulin, and insulin-like growth factor-I, and a tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), can also activate Ras under the same conditions. A tyrosine kinase-specific inhibitor, genistein, inhibited the increase of Ras.GTP induced by NGF and other factors. On the other hand, down-regulation of protein kinase C (PKC) by prolonged treatment with TPA, which sufficiently blocked TPA-induced Ras activation, did not abolish the formation of Ras.GTP by NGF. These results suggest that tyrosine kinases rather than PKC play a major role in the NGF-induced activation of Ras in PC12 cells.

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Set	Items	Description
S1	189065	NEURAL OR NEURONAL
S2	14575	CULTUR? AND S1
S3	3470	DIFFERENTIAT? AND S2
S4	727	GROWTH(N)FACTOR? AND S3
S5	126	SERUM AND S4
S6	86	RD S5 (unique items)

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